

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 2

IN THE CLAIMS:

The claims as currently presented and under consideration, are presented below for the Examiner's convenience and to comply with 37 CFR §1.121:

Claims 1-4 (Cancelled)

5. (Previously Presented) The pullulanase of Claim 6, wherein the pullulanase is obtained from a *Bacillus deramification* having the designation T89.117D in the LMG culture collection.

6. (Previously Presented) A truncated *Bacillus* pullulanase comprising a deletion of about 100 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

7. (Previously Presented) A truncated *Bacillus* pullulanase comprising a deletion of about 200 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

8. (Previously Presented) A truncated *Bacillus* pullulanase comprising a deletion of about 300 amino acids from the amino terminus of a pullulanase obtainable from *Bacillus deramificans*, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

9. (Previously Presented) A truncated *Bacillus* pullulanase comprising a deletion that is 98 amino acids from the amino terminus of *Bacillus deramificans* pullulanase, wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1, 6-glucosidic bond.

GC396-2AAF

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 3

10. (Previously Presented) A truncated *Bacillus pullulanase* comprising a deletion that is 102 amino acids from the amino terminus of *B. deramificans* pullulanase, wherein said truncated pullulanase is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

11. (Cancelled)

12. (Currently Amended) A modified *Bacillus pullulanase* which is capable of hydrolysis of an alpha-1,6-glucosidic bond, wherein the modification is an addition of at least one amino acid to the amino terminus of a mature pullulanase amino acid sequence obtainable from a *Bacillus deramificans*, wherein the additional amino acid at the amino terminus is an alanine.

13. (Cancelled)

14. (Previously Presented) A truncated *Bacillus pullulanase* produced by a method comprising the steps of

a) obtaining a recombinant host cell comprising nucleic acid encoding a mature *Bacillus pullulanase* said nucleic acid having at least 90 % identity to the polynucleotide sequence as shown in SEQ ID NO:1,

b) culturing said host cell under conditions suitable for the production of a truncated pullulanase, and

c) recovering the truncated pullulanase wherein the truncated *Bacillus pullulanase* comprises a deletion of about 100 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase, wherein said truncated pullulanase comprises a conserved Y region, and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

15. (Previously Presented) The pullulanase of Claim 14 wherein said host cell is *B. licheniformis* which comprises a first gene encoding Carlsberg protease and a

GC396-2AAF

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 4

second gene encoding endo Glu C protease, the first and/or second gene which codes for the protease(s) having been altered such that the protease activity is essentially eliminated.

Claims 16 - 26 (Cancelled)

27. (Previously Presented) An enzymatic composition comprising a truncated *Bacillus deramificans* pullulanase wherein said truncated pullulanase is selected from the group of pullulanases consisting of

a) a deletion of up to about 100 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase,

b) a deletion of up to about 200 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase, and

c) a deletion of up to about 300 amino acids from the amino terminus of a *Bacillus deramificans* pullulanase,

wherein said truncated pullulanase of a), b) or c) comprises a conserved Y position and is capable of catalyzing the hydrolysis of an alpha-1,6-glucosidic bond.

28. (Previously Presented) The enzymatic composition of Claim 27 wherein the truncated pullulanase has a deletion of amino acids from the amino terminus of up to about 100 amino acids.

29. (Previously Presented) The enzymatic composition of Claim 27 wherein the truncated pullulanase has a deletion of amino acids from the amino terminus of up to about 200 amino acids.

30. (Previously Presented) The enzymatic composition of Claim 27 wherein the truncated pullulanase has a deletion of amino acids from the amino terminus of up to about 300 amino acids.

GC396-2AAF

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 5

31. (Previously Presented) An enzymatic composition comprising the pullulanase of Claim 9, wherein the pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 99, a glutamic acid.

32. (Previously Presented) An enzymatic composition comprising the pullulanase of Claim 10, wherein the pullulanase has the amino acid sequence as shown in SEQ ID NO:2 beginning at amino acid residue 103, a glutamic acid.

33. (Original) The composition of Claim 27 further comprising an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.

34. (Original) The composition of Claim 27 further comprising a glucoamylase.

35. (Original) The composition of Claim 34 wherein the glucoamylase is obtainable from an *Aspergillus* strain.

36. (Original) The composition of Claim 35 wherein the *Aspergillus* strain includes *Aspergillus niger*, *Aspergillus awamori* and *Aspergillus foetidus*.

37. (Original) The composition of Claim 27 wherein said composition is in a solid form.

38. (Original) The composition of Claim 27 wherein said composition is in a liquid form.

39. (Currently Amended) The composition of Claim 27 wherein said composition comprises ~~comprising~~ at least 60% truncated pullulanase.

GC396-2AAF

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 6

40. (Currently Amended) The composition of Claim 27 wherein said composition comprises ~~comprising~~ at least 80% truncated pullulanase.

Claims 41 to 51 (Cancelled)

52. (Previously Presented) The truncated *Bacillus* pullulanase of claim 6, wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

53. (Previously Presented) The truncated *Bacillus* pullulanase of claim 7, wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

54. (Previously Presented) The truncated *Bacillus* pullulanase of claim 8, wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

55. (Previously Presented) The enzymatic composition of claim 27 wherein said deletion is from a pullulanase having the sequence shown in SEQ ID NO: 2.

56. (Previously Presented) The truncated *Bacillus* pullulanase produced according to the method of claim 14, wherein the nucleic acid sequence encoding the mature pullulanase is SEQ ID NO: 1.

57. (Previously Presented) The truncated *Bacillus* pullulanase produced according to the method of claim 14, wherein the mature pullulanase has the sequence shown in SEQ ID NO: 2.

GC396-2AAF

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 7

58. (Previously Presented) The truncated *Bacillus pullulanase* of claim 9, wherein the pullulanase is obtained from a *Bacillus deramificans* having the designation T89.117D in the LMG culture collection.

59. (Previously Presented) The truncated *Bacillus pullulanase* of claim 10, wherein the pullulanase is obtained from a *Bacillus deramificans* having the designation T89.117D in the LMG culture collection.

60. (Previously Presented) The truncated *Bacillus pullulanase* of claim 6, further comprising a conserved VWAP (SEQ ID NO:9) region.

61. (Previously Presented) The truncated *Bacillus pullulanase* of claim 7, further comprising a conserved VWAP (SEQ ID NO:9) region.

62. (Previously Presented) The truncated *Bacillus pullulanase* of claim 8, further comprising a conserved VWAP (SEQ ID NO:9) region.

63. (Previously Presented) The truncated *Bacillus pullulanase* of claim 14, further comprising a conserved VWAP (SEQ ID NO:9) region.

64. (Previously Presented) The truncated *Bacillus pullulanase* of claim 27, further comprising a conserved VWAP (SEQ ID NO:9) region.

65. (Previously Presented) The composition of Claim 31 further comprising an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.

GC396-2AAF

U.S. Serial No.: 09/262,126
Amendment dated May 17, 2005
Reply to Office Action of March 31, 2005

Page 8

66. (Previously Presented) The composition of Claim 32 further comprising an enzyme selected from the group consisting of glucoamylase, alpha-amylase, beta-amylase, alpha-glucosidase, isoamylase, cyclomaltodextrin, glucotransferase, beta-glucanase, glucose isomerase, saccharifying enzymes, and/or enzymes which cleave glucosidic bonds.

GC396-2AAF